“Molecular Docking and Simulation Studies on Phytochemical profile of *Capparis decidua* for inhibition of acetylcholinesterase in AD”

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SUBMITTED BY

SALEHA SIDDIQUI
2K21/MSCBIO/38

Under the supervision of

PROFESSOR YASHA HASIJA

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi college of Engineering)

Bawana Road, Delhi-110042

MAY,2023

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi college of Engineering)

Bawana Road, Delhi-110042

M.Sc Biotechnology

Saleha Siddiqui

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CANDIDATE'S DECLARATION

I, Saleha Siddiqui, 2K21/MSCBIO/38 of MSc. Biotechnology, hereby declare that the project Dissertation titled "Molecular Docking and Simulation Studies on phytochemical profile of *Capparis decidua* for inhibition of Acetylcholinesterase in AD" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi. in partial fulfilment of the requirement for the award of the degree of Master of Science is an authentic record of my own carried out work under the supervision of professor Yasha Hasija. The matter presented in this report has not been submitted by me for the award of any other degree of this or any other Institute/University. The work has been accepted in IEEE conference with the following details:

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Saleha Siddiqui

Place: Delhi

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi college of Engineering)

Bawana Road, Delhi-110042

CERTIFICATE

I hereby certify that the Project Dissertation titled "**Molecular Docking and Simulation Studies on phytochemical profile of *Capparis decidua* for inhibition of Acetylcholinesterase in AD**", which is submitted by Saleha Siddiqui, 2K21/MSCBIO/38, Delhi Technological University Delhi, in partial fulfilment of the requirement for the award of the degree of Masters in Science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

Place: Delhi:

Date:

PROFESSOR YASHA HASIJA
SUPERVISOR
Department of biotechnology
Delhi technological university

PROF. PRAVIR KUMAR
HEAD OF DEPARTMENT
Department of biotechnology
Delhi technological university

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ABSTRACT

Alzheimer's disease (AD) is a sustained neurological disorder that is characterized by declination in cognition and loss of memory. The emergence of lesions such as neuritic plaques, neurofibrillary tangles (NFTs), cerebral amyloid angiopathy, neuronal loss, and cholinergic deficiency are markers suggestive of AD. Hence, indicating the potential significance to target associated proteins like amyloid precursor protein cleaving enzyme, cholinesterase such as butyrylcholinesterase (BuChE), and acetylcholinesterase (AChE) for therapeutic intervention. Despite ongoing studies there is no successful treatment measures for AD. Thereby, it becomes of utmost important to exploit bioactive substance from natural sources to improve the existing therapies and treat AD effectively.

Capparis decidua a plant of medicinal importance from the family *Capparacea* contains abundance array of secondary metabolites like terpenoids, alkaloids, polyphenols,and phytosterols, which imparts various benefits like anti-inflammatory activitie, anti-diabetic, anthelmintic, and antioxidant properties. The aim of this preliminary research is to explore the possibility of bio compounds derived from *Capparis decidua* as a potential candidate for treatment of AD. In order to achieve this, an initial *in-silico* assessment was performed by first evaluating the medicative value of *Capparis decidua* for the amelioration of AD. The analysis involved evaluation of the binding affinities of 55 phytochemicals obtained from *Capparis decidua* to the target protein acetylcholinesterase, AChE with the help of detailed docking study using Autodock Vina followed by validation of molecular docking study with the help of molecular dynamics simulations using 2020.3 version of GROMACS. Results demonstrated that Linolenic Acid and 2-hydroxycinnamic Acid exhibited lowest binding energy of -7.8 (Kcal/mol) and -7.1 (Kcal/mol), respectively. Hence the highest binding affinities towards the target, AChE. These scores which were comparably equivalent to the binding energy of the standard FDA approved control drug, Donepezil (-7.0 Kcal/mol). Further the stability of the complex system was investigated using molecular dynamic (MD) simulations. MD simulations utilize measures including root-mean-square deviations (RMSD), root-mean-square fluctuation and Radius of gyration (Rg). Additionally, to this, various analyses were conducted to assess drug-likeness, bioactivity, permeation through the Blood-Brain Barrier (BBB), and bioavailability of the chosen phytochemicals, which hold the potential as therapeutic agents against neurodegenerative disorders like AD.

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LIST OF ABBREVIATIONS

1. AD – Alzheimer's Disease
2. Ach- Acetylcholine
3. AChE - Acetylcholinesterase
4. BuChE. - Butrylcholinesterase
5. RO5- Rule of five
6. NRL- Nuclear receptor ligand
7. ICM- Ion Channel Modulator
8. GPCR: G-protein coupled Receptor
9. EI- Enzyme Inhibitor
10. KI- Kinase Inhibitor
11. BBB- Blood Brain Barrier
12. AChEI - Acetylcholinesterase Inhibitors
13. BACE-1- β -site precursor protein cleaving enzyme-1
14. FDA- Food and Drug Administration
15. SAR- Structure activity relationship
16. NFT - Neurofibrillary tangles
17. APP- Amyloid precursor protein
18. NMDA- N-Methyl-D-aspartate Receptor
19. MAO – Monoamine oxidase
20. AEP: Aspargine endopeptidase
21. AIF- Apoptosis inducing factor
22. PARP- poly(ADP-ribose) polymerase-1
23. ChAT- Choline acetyltransferase
24. Acetyl-Co A- Acetyl Coenzyme A
25. VChAT- Vesicular acetylcholine transporter
26. CHT1- Choline transporter
27. BBB- Blood Brain Barrier
28. PDB- Protein Database
29. IMPPAT- Indian Medicinal Plants, Phytochemicals and Therapeutics
30. ML- Machine Learning
31. MD- Molecular Dynamics
32. PLIP- Protein Ligand Interaction Profiler
33. ADME- Absorption, Distribution, Metabolism, Excretion
34. SMILES- Simplified Molecular Input Line Entry System
35. RMSD- Root-mean-square Deviation
36. RMSF- Root mean square fluctuations

CHAPTER 1: INTRODUCTION

Neurodegenerative disorders are among the biggest challenges faced by public health, leading to dysfunctional nerve cells and consequently, progressive dementia. 75% of the dementia cases that affect individuals aged 65 and above, are caused by Alzheimer's disease [1] . The characteristic symptoms of the disease include memory loss, personality changes, abnormal behaviour and decreased cognitive abilities. The major risk factors contributing to the onset of AD include family genetics, traumatic brain injury, age related degeneration, inappropriate diet and cardiovascular disease. As the disease progresses, the outcome of this crippling disease can be fatal.

The pathophysiology of the disease is contributed to the accumulation of amyloid- β (Aβ) peptides, which form neuritic plaques extracellularly, as well as the accumulation of phosphorylated Tau protein, thereby causing neurofibrillary tangles intracellularly. Among the multiple proposed aetiologies and pathogeneses, the cholinergic hypothesis is the earliest and most extensively studied [2]. The hypothesis states that Acetylcholine (Ach), a neurotransmitter, plays an important role in the excitation of neurons, synaptic transmission and memory formation. The acetylcholine in the synapse is broken down into acetyl and choline with the help of a group of esterase, including acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) [3]. The pathogenesis of AD is contributed to dysregulated cholinergic system, which is due to deficiency of acetylcholine along with decreased cholinergic neurons in the hippocampus. Thus, acetylcholinesterase inhibitors (AChEIs) are a promising therapeutic modality to treat Alzheimer’s disease.

Other significant factors in the progression of AD include increased production of Aβ peptides by β -site precursor protein cleaving enzyme-1 (BACE-1), impaired signalling and abnormal synaptic transmission. While there are FDA-approved medications available for the treatment of AD, they only delay the onset of the disease [4]. This limitation has sparked an interest in discovering potent natural inhibitors targeting the proteins implicated in the pathogenesis of AD. Substantial research has already commenced, looking to overcome the limitations of existing drugs and consequently identifying new and effective compounds with better outcomes and decreased side effects [3], [5].

The objective of this study is to perform an *in-silico* analysis of phytochemicals extracted from *Capparis decidua*, as a potential alternative approach to inhibit acetylcholinesterase (AChE) and consequently alleviate AD symptoms. *Capparis decidua* belonging to the *Capparaceae* family, growing in the tropical and subtropical region, has shown remarkable medicinal characteristics. Its therapeutic nature is attributed to its phytochemical constituents including alkaloids, polyphenols, terpenoids and fatty acids. The application of phytochemicals derived from natural sources as unconventional therapeutic agents has gained popularity due to its availability and ease of extraction, potent pharmacological properties and low toxicity [6].

In this study, *an in-silico* analysis was conducted to investigate 55 phytochemicals extracted from *Capparis decidua*, using Autodock Vina. The selection criteria were based on binding affinities of the phytochemicals, comparable to that of Donepezil, a FDA approved standard AChE inhibitor used in the treatment of AD. The selected phytochemicals were then screened and assessed based on drug resemblance, bioactivity, bioavailability and adherence to Lipinski's rule of five. The overview of the methodology is show in in **Fig. 1.** The objective of this evaluation is to identify novel potential compounds for AD target intervention and to overcome the limitations of existing treatment modalities, and ultimately improve the existing approaches to manage AD [7], [8].

Valuable tools available in modern drug development include molecular docking and molecular dynamics (MD) for simulations were put to use, which helps in understanding the molecular interactions between potential drug candidates and their respective target proteins. These computational techniques aid in the rational designing of potent therapeutic agents by providing useful insights about the binding affinity, binding mode and stability of drug-protein complexes.

With respect to Alzheimer's disease (AD), molecular docking and MD simulations are significantly advantageous. These methods allow researchers to observe and study the interactions between phytochemicals, extracted from *Capparis,decidua* and the active site of acetylcholinesterase (AChE) in a virtual simulation. They also help in predicting the binding affinity of phytochemicals as well as identifying important key residues involved in the process. Furthermore, MD simulations provides a functional view of the drug-protein complex over time, which allows for observing conformational changes, protein

flexibility as well as intermolecular interactions. Molecular docking thus helps in selecting potential inhibitors for the further analysis.

MD simulations and molecular docking are significant in AD drug development as they help streamline the process of identifying and screening novel compounds. These methods can screen many phytochemicals and predicts their binding affinities, which can accelerate the drug discovery process and helps researchers focus their efforts and resources on the most promising candidates. Consequently, the time and resources utilised for in vitro and in vivo experiments is significantly reduced [9].

Additionally, the exploration of structure-activity relationships (SAR) of potential compounds is made possible using MD simulations. Using systematic modification of the chemical structure of phytochemicals and further assessment of their binding affinities, researchers can gain an insight into the major functional groups and structural features of said compounds which are crucial for efficient inhibition of AChE. As a result, this understanding can aid in designing and optimising novel drugs with improved and more potent pharmacological properties.

The utilisation of these computational tools can be used to revolutionise and accelerate the process of AD drug development which can in turn lead to targeted therapeutic interventions and more effective treatment modalities.

Objective of this study:

1. To investigate natural bioactive compounds as a substitutive therapeutic approach to ameliorate existing AD therapy
2. To analyse the drug likeness properties of the lead hits.
3. To evaluate the structural stability and deviations of the lead hits in complex with the target protein.

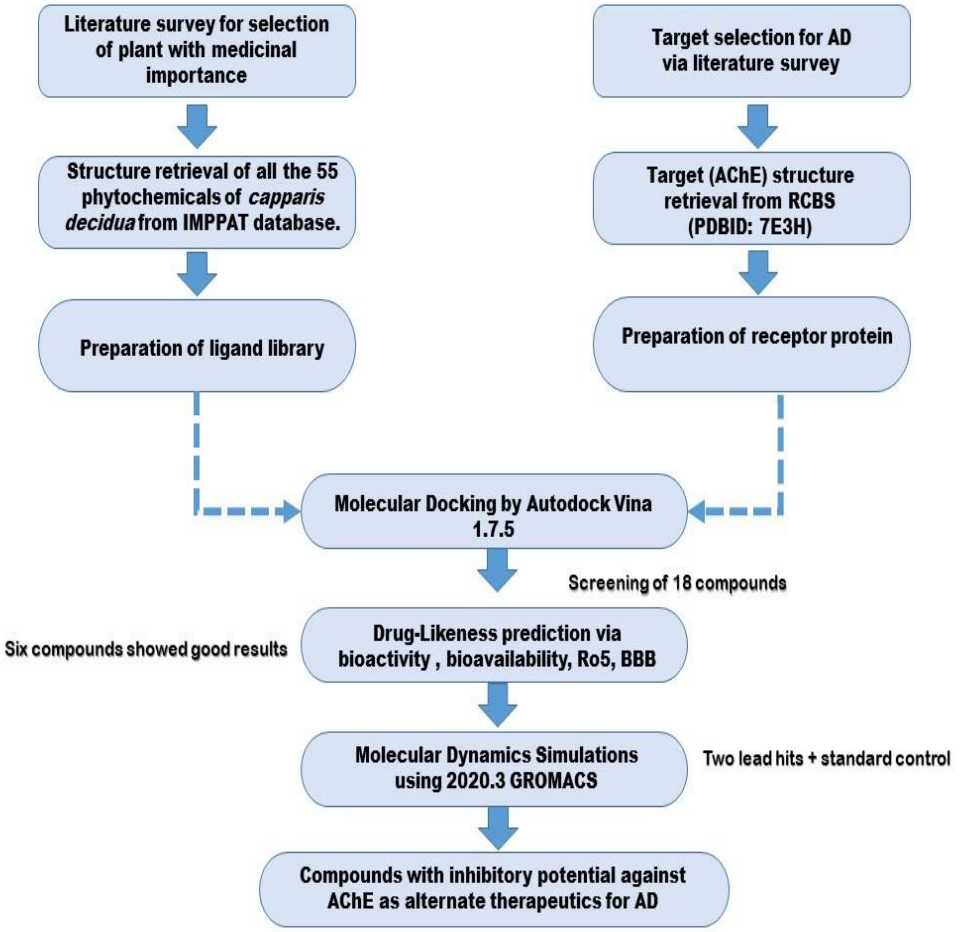

Figure 1: Overview of workflow of Methodology.

CHAPTER 2: LITERATURE REVIEW

2.1 Overview of Alzheimer's

Alzheimer's Disease is a complex neurological disorder that is prevalent among the elderly, especially those aged more than 65 years. According to the World Alzheimer's Report, 46.8 million individuals suffer from dementia all over the globe in 2015, and this figure is anticipated to increase almost by two folds every 20 years. It is also presumed that one new case of dementia develops every three seconds. Presently, 55 million people suffer from dementia worldwide, out of which 60% belong to low and middle-income nations [10]. There are around 10 million new cases each year, and it is estimated that, by the year 2050, the number will increase to 152 million cases of dementia worldwide. India reported 2 million cases of AD in 2020, and by 2050, 4.6 million cases are anticipated. There are 5.5 million AD sufferers in the United States alone, making it the most prevalent kind of dementia [11].

A familial type of AD, which affects people from 30 to 50 years of age, is linked to certain genetic abnormalities, has been proven to afflict a lesser percentage of AD patients (less than 5%). Further research into AD is needed, paying particular attention to the fact that women make up the majority of AD patients compared to males, according to recent studies. Recent research has revealed a startling pattern, showing that women are twice as likely as males to be impacted by AD. This striking disparity highlights the significance of investigating the underlying factors contributing to this gender difference in AD susceptibility [12].

Patients of AD experience a wide range of symptoms during the early stages of the disease such as difficulties remembering people, places, and recent events alongside to issues with short-term memory retention. Furthermore, mood fluctuations and a lack of capacity to comprehend and integrate new knowledge. With the progression of the disease to Late-stage, clinical symptoms intensify. These may include confusion, lowered judgement, and impaired communication abilities. In the final stages, people could experience challenges with walking, eating, and swallowing. Additionally, as the illness progresses, several metabolic problems might become apparent [13].

The actual aetiology of AD is unclear, but it is characterized by “A plaques” formation which is extracellular and also known “senile plaques”, which are mostly made of amyloid- (A) peptides. Additionally, neurofibrillary tangles (NFTs) consisting of tau-rich proteins are formed intracellularly, making these two elements important pathological markers of AD [14]. While another familial forms of AD (fAD) are caused by mutational alteration in genes including amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2), sporadic AD is still quite common and is impacted by a number of genetic variables. The familial type of AD has a genetic susceptibility that is caused by autosomal dominant linked mutations [15], [16].

Development of neurofibrillary tau tangles (NFTs) are also implicated significantly in the pathogenesis of Alzheimer's disease. NFTs are formed due to abnormal phosphorylation and accumulation of tau proteins. Destabilization of microtubules and disturbances in axonal transport are the results of this process, which eventually results in memory loss and neuronal death. These incidents show a connection between the cognitive impairment seen in AD and abnormal tau proteins [17].

Aβ peptide deposition was once thought to be the main factor contributing to the pathological alterations in tau protein function. However, current knowledge indicates that both processes can take place simultaneously, aggravating their negative effects and adding to the decline in cognitive function seen in Alzheimer's disease.

Evidence also suggests that due to its soluble and diffusible nature, tau oligomers may contribute to the spread of their detrimental effects all over the brain [18].

Amyloid cascade theory suggests that cleavage of APP leading to accumulation of Aβ peptides is a crucial step in the pathogenesis of AD. This cleavage is attributed to the enzymatic activity of β and γ-secretase. Due to the conformational changes occurring at the molecular level, the peptides resulting from the cleavage show an increase in the number of β -sheet structures. These conformational and structural changes make it possible for Aβ peptides to form aggregates and are consequently accumulated in the patient’s brain [19].

Various studies suggest that these plaques can store and sequester soluble oligomers, the build-up of which has been observed in patients with Alzheimer's disease. It is worth noting that the presence of these oligomers in synaptic regions is also observed in patient's experiencing memory loss symptoms. The development of dementia is also linked to

synapses undergoing pathological changes, which can be attributed to these sequestered oligomers. It is important to note that cognitive impairment in AD is not solely attributed to the accumulation of Aβ and abnormal tau proteins but is also influenced by the dysfunction of synaptic connections [20].

Aβ peptides have been identified to bind to cellular receptors and initiate a variety of signalling pathways, including calcium and oxidative signalling. They impairment in connections between neurons by interfering with synaptic plasticity receptors, leads to increase in release of glutamate neurotransmitter, which induces hyper phosphorylation of tau, leading to disruption of axon transport, and contributing to memory impairment and inhibition of long-term potentiation (LTP) [21]. Although a significant number of studies have been conducted to explain the process behind detrimental impact of Aβ peptides, the mechanism underlying its toxicity remains unclear.

The neurological dysfunctions associated with AD progression has been linked to several proteins besides cholinesterase such as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Other involved proteins which show significant role in progression and manifestation of AD are protein kinase, asparagine endopeptidase (AEP), N-Methyl-D-aspartate (NMDA) receptor, monoamine oxidases (MAO), asparagine endopeptidase (AEP) and beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) [22].

Additionally, extensive investigation of already existing literature has unveiled that hyper activation of poly(ADP-ribose) polymerase-1(PARP) plays crucial part in AD. This hyperphosporylation is caused due to oxidative DNA damage or cellular injury, it further leads to significant depletion of NAD+ and ATP within the afflicted neurons. Eventually causing parthanatos, a process which involves activation of apoptosis-inducing factor (AIF) resulting in neuronal death. Based on several findings from multiple studies targeting PARP-1 repression could potentially serve as an alternative treatment strategy for AD. Suppressing the activity of PARP-1 has potential to mitigate the adverse effects of parthanatos and offer therapeutic benefits to people suffering from AD [23]–[25].

2.2 AChE: The Key Target

The cholinergic functioning of the brain is regulated by the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Various publications have studied the role of AChE

with respect to Alzheimer's disease and about 1500 manuscripts indexed into the PubMed, particularly regarding treatment approaches involving AChE inhibitors (AChE-I). Acetylcholinesterase (AChE), a type of serine hydrolase, plays a central role in regulating the cholinergic activity of the brain. It acts on acetylcholine (ACh), an essential neurotransmitter and chemical messenger responsible for neuronal communication. AChE hydrolyses ACh into acetate and choline, thereby terminating neurotransmission in synapses [26]. Impairment of this cholinergic system leads to cholinergic dysfunction, contributing to the progression of AD, forming the basis of the" cholinergic hypothesis". While butyrylcholinesterase (BChE) shares similarities with AChE it serves a distinct purpose. BChE provides a countermeasure against organophosphates and hydrolyses butyrylcholine. Together, AChE and BChE regulate and maintain the cholinergic neurotransmission, and thus imparting a key role in normal functioning of the brain [27].

Abnormal amounts of AChE enhance the formation of Aβ peptides to form an amyloid-AChE complex, which is implicated in the pathogenesis of Alzheimer's disease [28]. Cognitive function relies on the cholinergic pathway, which in turn relies on the neurotransmitter acetylcholine. Degeneration of cholinergic neurons and decreased levels of Ach in the synapse lead to the pathogenesis of Alzheimer's disease. Hence, understanding the cholinergic pathway is crucial to develop successful interventions to treat the disease.

The cholinergic pathway involves the following steps:

1) Formation of Ach: Choline acetyltransferase (ChAT) combines choline and acetyl-coenzyme A (Acetyl-CoA) in the cytoplasm of presynaptic neurons, to form Ach.
2) Formation of synaptic vesicles: Ach is packed into synaptic vesicles with the help of vesicular acetylcholine transporter (VAChT). Synaptic vesicle formation helps in the release of Ach in the future.
3) Depolarization induced exocytosis: When the presynaptic neuron is depolarized, the electrical activity induces the release of Ach from the synaptic vesicles into the synaptic cleft. This process is known as exocytosis.
4) Receptor Binding: The released ACh can bind to the receptors on the post synaptic neuron, which can either be nicotinic receptors or muscarinic receptors.

Depending on the nature of the receptor, the response elicited can either be stimulatory or inhibitory, and thus impact neuronal communication.

5) Hydrolysis and reuptake: After its interaction with receptors, Ach is acted upon by the enzyme acetylcholinesterase (AChE) in the synaptic cleft. The resulting hydrolysis leads to the formation of acetate and choline. This choline is then taken up by the presynaptic neuron via high affinity choline transporter (CHT-1) and is recycled [29].

Impairment of the cholinergic system results in decreased amount of Ach, leading to the progression of AD and further cognitive decline. One approach to manage the symptoms and improve cognitive functions is to target this pathway using acetylcholinesterase inhibitors such as donepezil [30].

AChE is known to show highly specific catalytic activity towards ACh (80%) and is hence recognised as a high-performance cholinesterase. BuChE, substitute for AChE, is a non-selective cholinesterase, capable of hydrolysing ACh as well as butyrylcholine [3].

Research on the pathophysiology of the disease has shown that during the early stages of AD, AChE accumulates at a faster rate than BuChE, but as the disease progresses, BChE levels increase and substitute the function of AChE.

2.3 Existing Treatment Options

Existing treatment modalities target the enzyme cholinesterase thereby inhibiting the breakdown of acetylcholine and increasing Ach levels in the synapse, resulting in improved cognitive function as well as reduced psychological and neuropsychiatric symptoms. Some approved cholinesterase inhibitors include donepezil, galantamine, tacrine, rivastigmine, and memantine (a glutamatergic system modulator) [31].

Among these drugs, the only naturally sourced cholinesterase inhibitor is Galantamine. It belongs to alkaloid class of phytochemicals. It increases Ach in the brain by reversibly and competitively inhibiting acetylcholinesterase (AChE). The existing drugs for the symptomatic treatment of AD, demonstrate notable adverse reactions such as hepatotoxicity and gastrointestinal effects (including nausea, vomiting, and diarrhea), reducing patient compliance and adherence to drug regimens [32].

The study of any drug like compound including phytochemicals as an inhibitor for AChE in Alzheimer's disease critically depends on their permeation through blood brain barrier

(BBB). The BBB, as the name suggests, is a highly selective barrier between the brain and the circulating blood which tightly regulates the movement of ions and molecules into the brain. Comprehending the significance of BBB is important as it essentially determines the accessibility and distribution of drug-like compounds targeting AChE in brain. BBB is restrictive in nature, hence several potential therapeutic compounds face challenges passing the barrier and reaching target site. Therefore, if phytochemicals are potent as therapeutic agents for AD, it is essential to check for their ability to permeate BBB for their efficacy. By elucidating interaction between BBB and phytochemicals, insights into mechanism of brain entry, optimal dosing strategies for effective inhibition of target, bioavailability can be determined. Thereby, aiding the development towards novel therapeutic approaches for AD treatment [33].

Although synthetic agents have had success as potential multifunctional drugs against AD, challenges in terms of pharmacokinetics and drug safety remain significant. Unfortunately, currently available medications provide only symptomatic relief and do not halt the process of neurodegeneration, underscoring the importance of discovering novel drugs. Hence the need to discover alternative treatment options which can overcome the existing limitations is understated.

2.4 Research gap and limitations in existing strategy

Over the past 2 decades, understanding and treating AD has significantly advanced. Yet there still remains notable research gaps and constraints in current therapeutic strategy. One of the persistent key challenge lies in the failure to halt or counteract the underlying neurodegenerative mechanism causing AD. While current strategies attempt to aid the symptoms and slow down the progression of disease, they fall short in terms of long term success. Further, obstacles remain with development of novel drugs, as several promising candidates fail to demonstrate substantial results.

Another drawback is the lack of personalized treatment choices, as AD is complicated condition varying widely in its progression and manifestation among different cohort of individuals. Furthermore, there is a need for improved early methods of early diagnostic technique, as many individuals are diagnosed when the disease has progressed to later stages and caused considerable damage to the brain functioning and cognition. More

effective and targeted treatments for AD can be developed by bridging these research gaps and addressing the shortcomings in current therapeutic strategies [34].

2.5 Plant derived natural compound as a potential alternative

Natural medicines derived from plants can offer to be a potential substitute in treatment of complicated neurodegenerative illnesses, owing to the safe and efficacious pharmacological nature of these compounds. However, the complex interaction of biological processes and proteins implicated in the pathogenesis of AD along with the complexity of the affected organs (particularly the brain), provide significant hurdles in identifying new and effective treatment modalities for Alzheimer's disease (AD) [35].

The curative potential of plant derived natural products and their bioactive compounds in treating different neurodegenerative disorders such as AD have been the subject of intense research recently. Despite the fact that there is still limited scientific knowledge about the effectiveness of phytomedicines, they have gained acceptance as a substitutive treatment approach due to their affordability, low toxicity, simple accessibility, and very few side effects. The growth in interest in exploiting phytochemicals has led to evaluating their potential bioactivity and nutritional value Many literature sources discuss the traditional usage of plants as medicines, and the molecular structures derived from these plants serve as the foundation for many contemporary drugs [36].

The densely branched, sturdy xerophytic climbing shrub *Capparis decidua* (Forssk.), also known as *C. aphylla*, belongs to the *Capparidaceae family.* Its natural habitat where it is primarily found mostly lies in subtropical and tropical regions. Locally it's called by a variety of names, including Kari, Kabra, Delha, Karil,Caper, Karyal, and others. It has been used in traditional medicine to treat a diverse spectrum of disease such as respiratory ailments (asthma, cough), gastrointestinal (dysentery, hepatitis, diarrhea, constipation, piles, ulcers), musculoskeletal problems, rheumatic diseases, renal disorders, cardiac disorders and dermatological lesions [9]. *C. decidua* is a plant with versatile characteristics and number of benefits. It has numerous useful properties such as those of digestive aid, menstrual cycle regulator, detoxifying agent, general well-being booster, aphrodisiac, and painkiller. Additionally, it has also been used as an herb in cooking to aid bowel motions and encourage appetite. Beyond its pharmacological advantages, *C. decidua* possesses a wide range of exceptional qualities also like abundance of essential

mineral components which boosts plant its nutritional value and establishes it as a great source of nutraceuticals.

A close examination into *C. decidua* revealed the existence of sitosterols, plant sterols with potential health benefits. These substances have antioxidant properties that efficiently counteracts dangerous free radicals generated in body. Contributing more to the medicinal potential. Plant also has polyamines, aliphatic components, diterpenic ester, and diterpene alcohol [37]

Furthermore, the bioactive chemicals present in *C.decidua* are shown to be vital for the growth, development, and proliferation of mammalian cells. This further emphasizes the plant's relevance in both conventional and modern medical practices by highlighting their potential intervention in a variety of biological processes can be put in use as a promising alternative treatment by targeting acetylcholinesterase (AChE) in AD [6], [37].

2.6 Molecular Docking

In the early phases of drug development, computationally driven research design known as molecular docking aids assessing of the potentially selected candidates, targeting on disease-associated proteins. Modern researchers use advanced and complex computer algorithms to efficiently choose the ‘hit’ or ‘lead’ candidates [38].

Predicting how a tiny chemical serving as a ligand will interact and bind to the active site of the given target protein is identified by this computational process. Molecular docking uses prediction algorithms to check how the ligand and protein will interact to create stable complexes by modelling their three-dimensional structures. This results in evaluation of their binding affinities and interacting mechanism [39]. The docking system gives bounded ligands a score functions by which indicates their binding affinity. Greater the binding affinity represents a lower docking score and vice versa. In the initial phases of drug discovery, molecular docking plays crucial role for identification of lead compounds, optimizing their chemical structures, and aiding further experimental studies. Utilizing *in silico* methods for the prediction of best potential drug candidates is efficient, affordable, and less prone to errors, thus saving time for further analysis [40]. But before proposing therapeutic candidates for human use, comprehensive experimental and pharmacological research are necessary [41]. Molecular docking is merely used as an early guiding tool in modern drug discovery. There are various tools used for Molecular docking and result analysis such as Autodock Vina,

The objective of this investigation of the non-mutated crystalized acetylcholinesterase (AChE) protein. The unique identification of the target AChE protein structure (7E3H) was supplied by the Protein Data Bank (PDB) through specific identification number. The crystal structure of human AChE complexed with the FDA-approved medication Donepezil was retrieved as a control to set a standard control.

The 7E3H structure comprises of two distinct chains A and B which are required for the protein to function. The 540 amino acids that make up AChE molecule individually contribute to its structural and functional characteristics. Furthermore, ligand as a small molecule (E20) consisting of chains E and F was identified as in the complex structure of 7E3H. The molecular weight of the structure was 1AUTO7 kDa suggesting its size and complexity.

The FDA-authorized second-generation cholinesterase inhibitor donepezil, which is the only selective AChE inhibitor applied for treating varying severities of Alzheimer's disease (AD), served as the study's positive control. It is also often prescribed to regulate AD associated dementia. It acts on AChE to reversibly block acetylcholinesterase enzyme and inhibit its action, which further prevents synapse loss and neurodegeneration associated processes [42].

Donepezil also has an impact on expression of BACE1, according to several research. For instance, Sarno Alveis found that patients who were prescribed donepezil on long term basis showed a substantial decrease and downregulation in expression of BACE1 protein expression, inferring a possibility for disease modification [43]. Additionally, it is apparent that several analogs of donepezil have inhibitory effect on BACE1 [44].

Henceforth, Donepezil was employed as a standard to compare the binding scores of various phytochemicals from *Capparis Decidua* to bind to the target protein AChE (PDB: 7E3H).

2.7 Molecular Dynamics Simulation

Molecular dynamics (MD) simulation has emerged as a valuable *in silico* method for validation of molecular docking studies. MD simulations approaches a possibility to investigate the dynamic behavior of biological systems extensively by modelling the movement and interactions of atoms and molecule over a time frame, allowing exploration of structural dynamics and conformational changes in target protein. MD simulation give insights on stability, interaction and aggregation process of the target

protein, which are essential for understanding the characteristic pathological features such as amyloid plaque and neurofibrillary tangles (NFT). It also helps in exploration of binding interactions between target protein associated with AD such as acetylcholinesterase (AChE) and prospective therapeutic compounds like phytochemicals.

Thereby, by simulating the behavior of these molecules MD simulations serve as powerful computational tool to elucidate the fundamental mechanism at molecular levels that leads to AD and aid the rational development of novel strategies by modeling behavior of biological systems [45].

CHAPTER 3: MATERIALS AND METHODOLOGY

3.1 Selecting and refining receptor protein

The RCBS Proteins Database served as a web tool for recognizing the gene encoding for our target protein, AChE. The database is designed to serve as an extensive resource aiding researchers to acquire data relevant to their research studies. Properties and characteristics like protein structure, sequence, functional annotations for various protein can be explored and analysed. The crystalized structure of human AChE coupled with its ligand E20 (CID 3152) was retrieved under the unique PDB ID: 7E3H in PDB format. An accepted standardized format that represents 3D coordinates of atoms as well as information like type of atom, metadata and bond connectivity [46], [47].

BIOVIA Discovery Studio (DS Visualizer Client Windows 64 bit) a complex software application designed for molecular modelling and simulation, was employed to get rid of water molecule, heteroatoms, ligands from the protein structure while adding polar hydrogen. By using SBD site sphere and the ligand associated with the complex the x, y, z coordinates of the centre Grid Box were presumed as -43.368, 37.722, -30.313, respectively. Now the protein which is prepared was obtained in PDB format.

A widely used software for molecular docking investigations and virtual screening, Autodock Vina 1.7.5 [48] was utilized for addition and distribution of Kollman's charges. Atoms were designated AD4 types after which the PDB format was converted into PDBQT format.

3.2 Preparation of phytochemicals as ligand library using IMPPAT

Donepezil (ZINC597013), which served as a control inhibitor of AChE was downloaded in format of SDF using the ZINC database. To proceed with further analysis, it was translated to the format of PDBQT with the aid of Open Babel GUI [49]. It is a versatile chemical toolkit designed for chemical data functionalities including data storage, analysis, conversion and data search. It allows users to modify and convert chemical data across multiple formats.

Additionally, another manually computerized database for phytochemicals of Indian medicinal plant, IMPPAT was employed to generate a library of 55 phytochemicals derived from *Capparis decidua*. Some of these phytochemicals are shown in **Fig. 2**. The files were extracted from IMPPAT in PDB format [21]. Each phytochemical from the library was individually translated to PDBQT format by Open Babel GUI [49]. This conversion in PDBQT format enables compatibility with software and programmers used in molecular docking research for drug discovery.

3.3 Molecular Docking of Protein and Ligand using Autodock vina

For *in-silico* docking experiment, Autodock Vina 1.7.5 was employed. The canter grid coordinates were set to X = -43.368, Y = 37.722, Z = -30.313, and X, Y, Z sizes to 100, 100, 110, respectively. These coordinates determine the active site search domain for binding of ligand to the target protein AChE. The config. file was created with the given grid parameters. The docking settings were defined by setting num mode and energy range to 10 and 4, respectively. These parameters determine the total number of docking pose to be created for each ligand and energy range for acceptable binding affinities. Autodock Vina was used to perform docking calculation of all ligands, including both standard and phytochemicals, against the target protein AChE, and their respective binding affinities were calculated in Kcal/mol using PERL. Estimation of binding affinities for individual ligands was simplified using the PERL programming language.

3.4 Docking Interaction analysis via PLIP

Several web tools and software including PyMol, PLIP, as well as BIOVIA Discovery Studio, were used to carry out docking interaction analysis on the log files and out files generated after molecular docking run [50], [51]. These tools were used to recognize and locate the amino acid sequence of target protein to which the ligand binds. To access the binding affinities of phytochemical ligands their free binding energy were contrasted with those of the standard reference inhibitor (Donepezil). Binding energy of ligand is indicative of its binding affinity, the phytochemicals with the lowest binding energy indicated strongest interaction and hence high binding affinity. Phytochemicals with lowest binding scores were chosen for further analysis of their pharmacokinetic properties.

3.5 Virtual Screening of pharmacokinetics and drug likeness of selected phytochemicals

The phytochemicals which had binding energy equivalent to those of standard inhibitors (Donepezil) were considered for further analysis involving the physiochemical and ADME profiling of ligands.

Lipinski's RO5 analysis: The RO5 analysis is performed to evaluate oral activity potential of selected phytochemical candidates. It only allows for a maximum of one infraction in their five inclusion requirements for orally taken active medication. The inclusion criteria are as for molecular mass to be lower than 500 Dalton, preferring small size molecule. Second inclusion criteria are for measure of lipophilicity, which is determined in terms of partition coefficient represented by LogP value, must be lower than 5. It gives a reference about the tendency of the compound to partition between lipophilic organic phase and aqueous phase. The range accepted for Molar refractivity should lie between 40-130. The molar refractivity range provides an estimate about the total size of molecule and its polarizability. Both H-bond donor and acceptor should also be less than 5 and 10, respectively. These two criteria are necessary as it relates to the ability of compound to associate with other molecules by forming H-bonds and the capacity of the compound to accept H-bonds from other molecules. The PDB files of selected phytochemicals were uploaded to an online tool and subjected to Lipinski's Rule of five to analyse their potential oral action [52].

In-silico Bioavailability analysis: This analysis dictates the extent and pace at which a medication reaches its intended biological target to show its functioning effect. For the assessment of bioavailability, it is crucial to comprehend pharmacokinetics features like absorption, distribution, metabolism, and excretion (ADME). The Simplifies Molecular Input Line Entry System, (SMILES) nomenclature were used to represent the selected phytochemicals. The SMILES notions were subjected to web tools like SwissADME and admetSAR [53]. These web tools are uniquely designed to estimate ADME through predictive models and algorithms [53].

Bioactivity score analysis: It analyses drug's binding interaction with certain target classes in the body, which is critically required to create functional medicine with higher binding selectivity and reduced side effects. The compounds as Nuclear receptor ligand (NRL) interacts with nuclear receptor which plays a key role in regulation of gene expression.

As kinase inhibitor (KI) these compound can modulate signalling pathways which involve kinase enzymes. As Enzyme Inhibitor (EI) these compound can inhibit crucial enzymes involved in biochemical pathways. As GPCR ligand they can modulate GPCR signalling pathways involved in multiple cellular process, as ligands for Ion channel modulator (ICM), they can regulate ion flux across membranes, and as Protease inhibitor (PI), they regulate the activity of proteases. The canonical SMILES notion of selected candidates was given to Molinspiration tool as input data for estimation of bioactivity scores. Bioactivity is crucial in determining the druggability of selected candidates as ligands for specific targets [54].

3.6 Molecular Dynamics (MD) Simulations using GROMACS

For further comprehensive evaluation to gain insights on the effect of structural alteration on the selected protein-ligand complexes including AChE-Donepezil (standard control), AChE-Linolenic Acid and AChE-2-hydroxycinnamic Acid Molecular dynamics simulations were performed using 2020.3 version of GROMACS. MD simulation analyses the conformational stability, interaction profile of the lead candidate with the target protein, thermodynamic properties of ligand-protein complex [6]. GROMACS, a licensed software intends to simulate the dynamics of biological molecules in diverse conditions using algorithms based on Newton's equation of motion. The PDB structures of the complexes after molecular docking was retrieved to serve as input to the CHARMM-GUI interface [55]. The system which aids in generation of input files for MD simulations in aqueous solvent condition was constructed using Solution builder. Force field of the ligand was parameterized using PDB coordinates. Rectangular type water box with 10 Å edge distance was set as one of the parameters. With the help of all-atomic force field CHARMM36 topology and parameter files were automated for the system. Neutralization of individual system was done by adding K^+ cations and CL^- anions at 0.15 conc. Dynamic input generation temperature was set at 303.15K. CHARMM GUI generated an output file in the format of ".tgz" for the GROMACS simulations. For the alleviation in steric hindrance of optimization of original structure energy minimization step was performed by setting nsteps to 5000000. Without any constraints, the duration of simulation for all the systems were set to 50 nanoseconds (ns). Analysis of the trajectory files were done using command-line interface to evaluate

RMSD (root-mean-square-deviation), RMSF (root-mean-square-fluctuation), Radius of gyration, Hydrogen bond plot, and various energies.

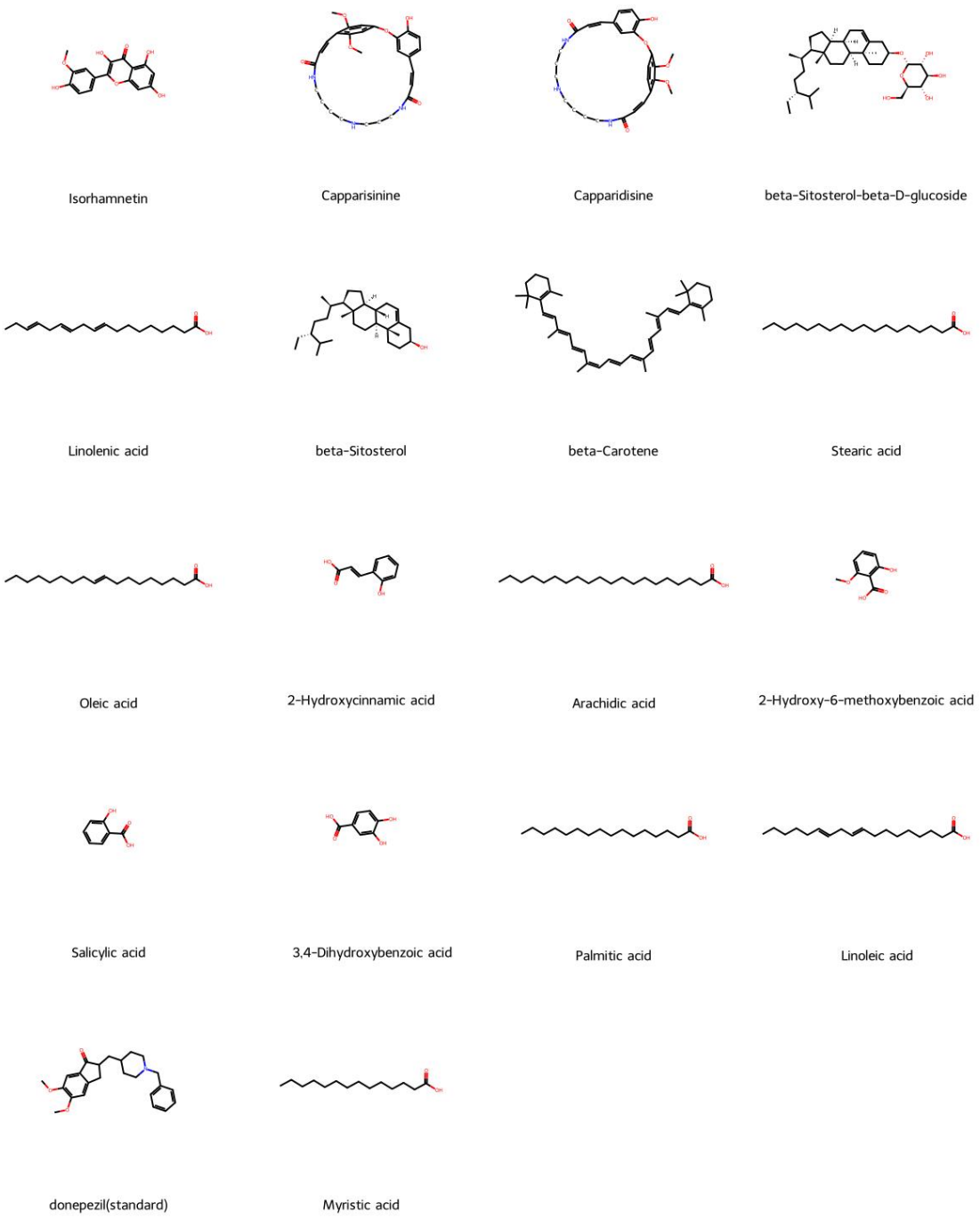

Figure 2: Structure of selected phytochemicals from *Capparis Decidua*

CHAPTER 4: RESULTS

4.1 Docking Analysis

Results obtained from this molecular docking study, which focused on the evaluation of the interaction between phytochemicals from *capparis decidua* and Acetylcholinesterase (AChE), reveals that natural substance as biomolecules can show inhibitory effects on the enzyme by binding to its active site residues. **Table I** depicts 18 out of 55 selected phytochemicals obtained from *Capparis decidua*, had binding affinity closely comparable to the binding affinity of the standard inhibitor also depicted in **Fig. 2**. Six phytochemical candidates shown in **Fig. 3 and Fig. 4**, were considered suitable on the basis of their binding energy for further analysis of pharmacokinetic properties. Two out of them stood to be the lead compounds, Linolenic Acid (-7.8 Kcal/mol) and 2-hydroxycinnamic Acid (-7.1 Kcal/mol), had the least binding energies and hence demonstrated the strongest affinities towards AChE, fit for its inhibition. The two selected lead compounds had binding affinities that are equivalent to that of the FDA authorized inhibitors of cholinesterase, Donepezil (-7.0 Kcal/mol) [4]. These results were validated using the literature of ongoing research with the help a machine learning based web tool called SwissTargetPrediction [56]. The prediction tool revealed that the same set of phytochemicals can potentially downregulate PARP-1 in AD [23]–[25].

The phytochemicals were subjected to ADME profiling for the evaluation of their pharmacokinetic properties. **Table I** displays phytochemical compounds exhibiting binding energies similar to a standard inhibitor along with their type of interaction with the amino acid residues. Among these compounds, six lead hits namely Linolenic Acid, Linoleic Acid, Palmitic Acid, 2- hydroxycinnamic Acid, Myristic Acid and Salicylic Acid, showed interaction with the amino acid residues of key target protein, AChE. The compounds shown in **Fig. 3 and Fig. 4**, demonstrated the ability to pass the blood brain barrier (BBB), which serves as a protective barrier separating the brain from the bloodstream. For further analysis of the pharmacokinetic characteristics the selected phytochemicals were subjected to ADME data evaluation carried using SwissADME [53]. The preferred compounds were assessed for parameters like BBB permeability, bioavailability score, and Lipinski RO5 infractions. The findings indicated that these

phytochemicals showed favourable properties fitting these parameters. The phytochemical permeating the blood brain barrier (BBB) are shown as positive in **Table II.**

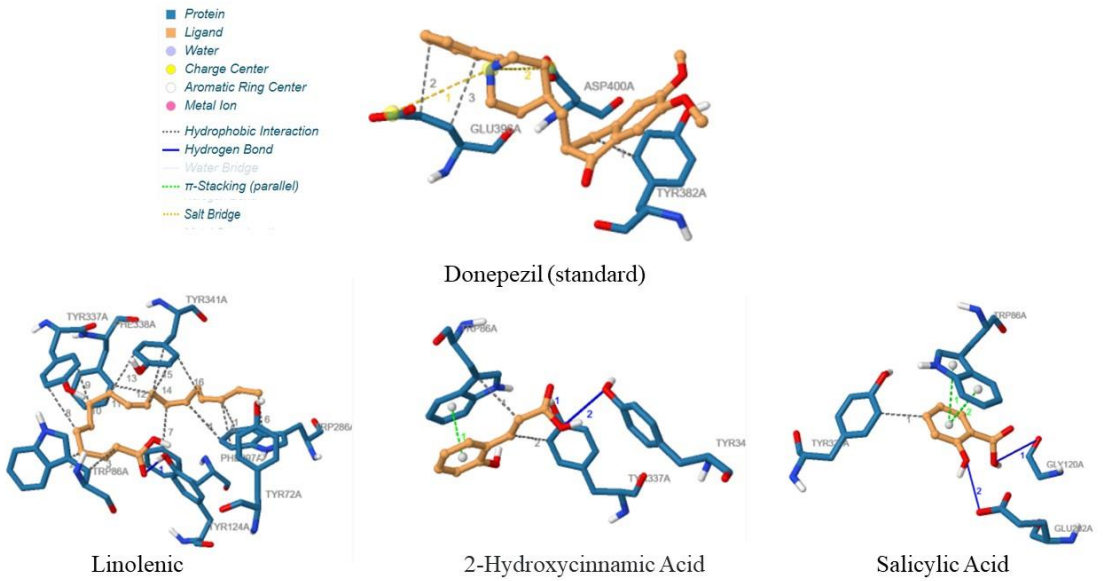

Figure 3: Structure and Interaction of Donepezil, Linolenic Acid, 2-hydroxycinnamic Acid with the target AChE.

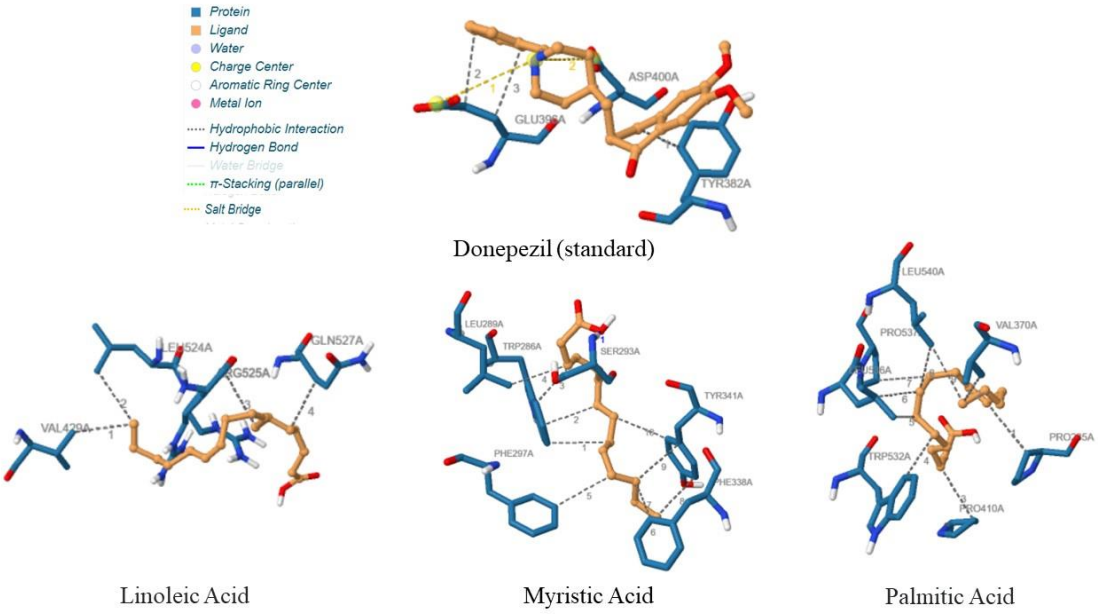

Figure 4: Structure and Interaction of Donepezil, Linoleic Acid, Myristic Acid Palmitic Acid with the target AChE.

Table I: Phytochemicals with their binding affinities and interacting residues

Ligand	Binding Energy (kcal/mol)	Interacting Amino Acids
Isorhamnetin	-9.4	Hydrophobic Interactions: PHE 297, TYR 341 H bonds: TYR 72, PHE 295 pi-stacking: TRP 286, TYR 341
Capparisinine	-9.1	Hydrophobic Interactions: TYR 72, PRO 537, LEU 540, LEU 76, TRP 86, GLN 527, ALA 528, VAL 239, ARG 524, GLU 243, GLU 296, ARG 246, VAL 408 TRP 286, TYR 382, LEU 289, VAL 330, TYR 337, LEU 524, PHE 338, TRP 532, TYR 341, , VAL 429 H bonds: TYR 72, GLY 120, TYR 124, ARG 525, SER 125, ASP 400, GLU 431, TYR 133, GLU 202, HIS 287, PHE 295, ALA 526, GLN 527, LYS 332, TYR 337, TYR 382, Salt bridges: LYS 332, ASP 400, ARG 525
Capparidisine	-8.9	Hydrophobic Interactions: GLN 527, ALA 528 H bonds: TYR 382, ASP 400, ALA 526, GLN 527
beta-Sitosterol-beta-D-glucoside	-8.3	Hydrophobic Interactions: TYR 382, GLN 527, GLN 527H bonds: ARG 525
Linolenic acid	-7.8	Hydrophobic Interactions: TYR 72, TRP 86, PHE 338, TYR, TRP 286, PHE 297, TYR 337, 341 H bonds: TYR 124
beta-Sitosterol	-7.8	Hydrophobic Interactions: LEU 289, TYR 341, TYR 72, LEU 76, TRP 286,H bonds: HIS 287
beta-Carotene	-7.5	Hydrophobic Interactions: VAL 239, GLU 243, ARG 246, TRP 532, PRO 537, LEU 540
Stearic acid	-7.2	Hydrophobic Interactions: TRP 86, TRP 286, PHE 338, TYR 341
Oleic acid	-7.2	Hydrophobic Interactions: TYR 72, TYR 341, TRP 86, TRP 286, PHE 338, PHE 297, TYR 337 H bonds: TYR 124, SER 125
2-Hydroxycinnamic acid	-7.1	Hydrophobic Interactions: TRP 86, TYR 337 H bonds: TYR 341, TYR 337 Pi- stacking: TRP 86
Arachidic acid	-7	Hydrophobic Interactions: TRP 286, TRP 86, TYR 337, TYR 72, PHE 338, TYR 341 H bonds: TYR 124, SER 125
Donepezil	-7	Hydrophobic Interactions: GLU396A, ASP400A, TYR382A.
2-Hydroxy-6-methoxybenzoic acid	-6.9	Hydrophobic Interactions: VAL 330, VAL 408, VAL 429, LEU 524, ARG 525 H bonds: LYS 332, GLU 431, ARG 525 Salt Bridges: LYS 332, ARG 525
Salicylic acid	-6.6	H bonds: GLY 120, GLU 202 pi-stacking: TRP 86
3,4-Dihydroxybenzoic acid	-6.3	Hydrophobic Interactions: TYR 337 H bonds: GLY 120, TYR 124, GLU 202, TYR 337 Pi-stacking: TRP 86
Linoleic acid	-5.7	Hydrophobic Interactions: VAL 429, LEU 524, ARG 525, GLN 527
Myristic acid	-5.6	Hydrophobic Interactions: PHE 297, PHE 338, TYR 341, LEU 289,H bonds: SER 293
Palmitic acid	-4.8	Hydrophobic Interactions: PRO 410, TRP 532, LEU 536, PRO 235, PRO 537, VAL 370, LEU 540

Table II.: Blood Brain Barrier pearmeability of selected phytochemicals

Phytochemical	BBB +/-
Linolenic Acid	Positive
2-HydroxycinnamicAcid	Positive
Salicylic Acid	Positive
Linoleic Acid	Positive
Myristic Acid	Positive
Palmitic Acid	Positive

Under Lipinski's rule, a compound should have molecular mass lower than 500 Daltons, range of molar refractivity to fall between 40-130, logarithmic octanol/water partition coefficient representing lipophilicity in terms of LogP to be less than 5, number of hydrogen bond donors and acceptor to be less than or equal to 5 and 10 respectively. At most only one infringement of these 5 criteria makes the compound fit to be orally active [57].

In accordance with the criteria of Lipinski's rule of 5 (RO5) **Table III** illustrates the assessment of selected phytochemicals, zero violations of RO5 were depicted by Myristic Acid, 2-hydroxycinnamic Acid, and Salicylic Acid. This implies that these compounds possess the properties that aligns with the criteria for oral bioavailability. However just one violation is observed for Linoleic Acid, Palmitic Acid, and Linolenic Acid. Despite this tiny deviation they still meet the general criteria of not more than one infringement, thus passing all six compounds through the rule of Lipinski.

Table III: Analysis of Lipinsk's Rule of Five (RO5)

RO5 analysis	Linolenic Acid	2-Hydroxycinnamic Acid	Salicylic Acid	Linoleic Acid	Myristic Acid	Palmitic Acid
Molecular Mass	280	164	138	280	228	265
Hydrogen Bond Donor	1	2	2	1	1	1
Hydrogen bond Acceptor	2	3	3	2	2	2
LogP	5.8845	1.49	1.0904	5.8845	4.7721	5.5523
Molar Refractivity	86.993774	44.77659	35.066097	86.993774	68.713783	77.9477 77

Table IV displays the score of bioactivity for the selected phytochemicals which were generated using a web tool Molinspiration. These scores represent the interactions of selected phytochemicals as ligand with various receptors like GPCR (G-protein coupled receptor) ligand, nuclear receptor ligand (NRL), protease inhibitor (PI), enzyme inhibitor (EI), ion channel modulator (ICM), and kinase inhibitor (KI) to produce specific pharmacological effects.

Table IV: Bioactivity scores of six selected phytochemicals

Phytochemical	GPCR	PI	EI	NRL	KI	ICM
Linolenic Acid	0.33	0.13	0.42	0.35	-0.19	0.23
2-HydroxycinnamicAcid	-0.64	-0.9	-0.21	-0.25	-0.97	-0.37
Salicylic Acid	-0.98	-1.14	-0.41	-0.79	-1.22	0.43
Linoleic Acid	0.29	0.12	0.38	0.31	-0.16	0.17
Myristic Acid	-0.11	-0.19	0.13	-0.06	-0.51	0.03
Palmitic Acid	0.02	-0.04	0.18	0.08	-0.33	0.06

Furthermore, an additional online tool is put to use for estimation of bioavailability scores. These score represents the percentage of compounds anticipated to enter systemic circulation based on the factors such as absorption, metabolism and excretion. **Table V** enlists likelihood of potential compounds to reach their desired target site and show therapeutic effects calculated in terms of bioactivity.

Table V: Bioavailability Scores of Six selected phytochemicals

Phytochemical	Bioavailabilityscore
Linolenic Acid	0.55
2-HydroxycinnamicAcid	0.85
Salicylic Acid	0.85
Linoleic Acid	0.55
Myristic Acid	0.85
Palmitic Acid	0.85

4.2 Molecular Dyanamics Simulation to investigate best docked compound

Three complexes were selected for MD simulations, one of these three complexes was standard control, Donepezil in complex with the target protein, AChE (AChE-Donepezil) as complex 1. The other two complexes were the lead phytochemicals which showed comparable binding energies to the control, Linolenic Acid (-7.8 Kcal/mol) and 2-hyroxycinnamic Acid (-7.1 Kcal/mol), in form of AChE-Linolenic Acid and AChE-2-hydroxycinnamic Acid as complex 2 and complex 3 as shown in **Fig.5.** For the accuracy of MD simulations multiple trajectories underwent examinations, including thermostats, pressure, energy, density. The temperature was kept constant at 303.15 K through the span of 50ns simulation time. Average value of pressure was held constant at 1 bar. Monitoring of these parameters, helped us to validate the stability and accuracy of the simulation system ensuring the desired environment was maintained throughout the simulation time.

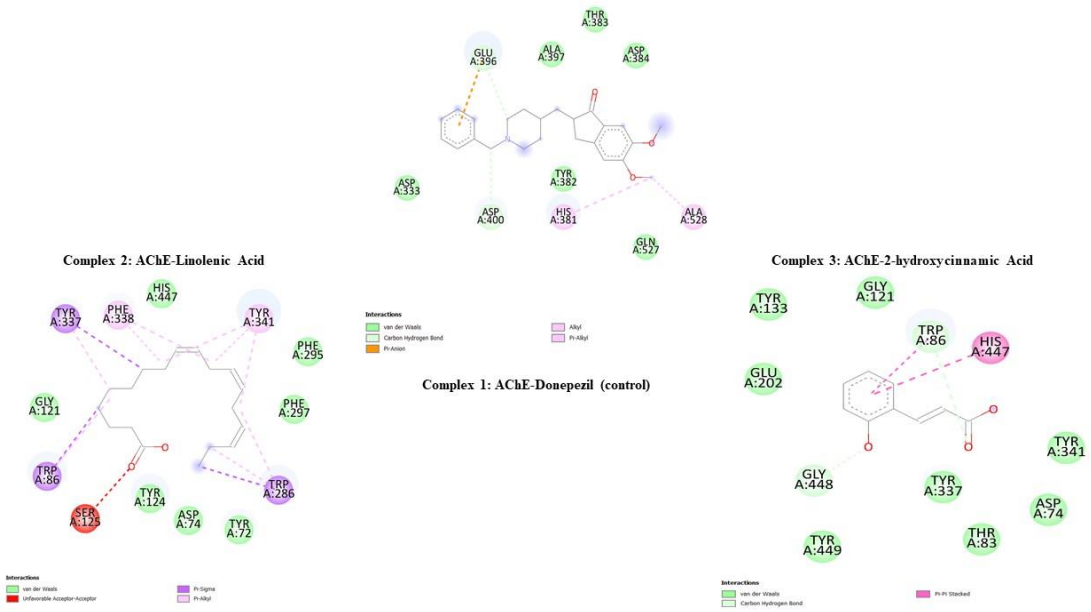

Figure 5: 2-D Interactions of two lead hits and Donepezil with their target AChE

4.3 Analysis of Root-Mean-Square Devations (RMSD)

To accesses the conformational dynamics and structural stability of protein ligand complexes up to 50 ns, MD simulation was performed to compute Root mean square deviation value. RMSD estimates the average deviation of the atoms in simulated structure in contrast with the reference structure, which is often the original bounded state

of protein-ligand complex. RMSD values give an insight of structural alteration and flexibility of the complex. Higher RMSD values are indicative of more deviation and flexibility, whilst lower RMSD values are suggestive of more stability and less deviation. RMSD estimation in MD simulation of a protein-ligand complex aids in characterization of potential energy changes and binding interactions in the dynamic state. Henceforth the results of RMSD analysis provide insights for rational understanding of drug designing techniques, mechanism of action of biological molecule within a system. Complex's dynamic behaviour can be comprehended by RMSD graphs as shown in **Fig. 6**. The command "gmx rms" is used for the generation of. xvg file for RMSD plots [58].

For complex 1, RMSD values at the 0-1 ns is relatively low indicating initial stability, at 4 ns some moderate range fluctuations can be observed indicating minor structural alteration or dynamic changes, a stable plateau is attained around 4-6 ns. There is a sudden peak in RMSD values reading in the range of 1.0-1.2 nm around 6.5 ns depicting significant structural alteration. The flexibility of the structure deviates highly between 8.5-10.5 ns, RMSD values falling in the range of 4.25-4.44 nm and then gradually transitions toward more stable conformation afterwards. In the later stages, beyond 14 ns there is a span of increase in peak of RMSD values indicating potentially persistent but minor fluctuations until the end of 50 ns. Overall, the graph depicts both stable and dynamics stages of complex 1.

For complex 2, the graph starts from a very low RMSD values (0,.000014) initially, indicating stable conformation of the complex, from 0.1 to 1 ns there is a gradual increase in the RMSD values depicting minor fluctuation in structure of the complex. As the simulation proceed from 1-10 ns the values remains constant reading 0.5nm on RMSD scale indicating significant deviations. A sudden drop in RMSD value (0.486) is seen at around 10.5 ns. From there onward until 20 ns fluctuation in RMSD value indicates flexibility within the complex. In the range of 20-30 ns a minor increase RMSD value shows structural fluctuations. After 30 ns RMSD shows continuous fluctuations depicting ongoing rearrangements in the complex 2. RMSD values for complex 3 is suggestive of some periodic fluctuation over the span of 50 ns simulation run. Initially at 0 ns RMSD value reads to be at 0.00050, confirming to a stable conformation of the complex. As the time proceeds the RMSD values increases to 0.3 nm to 0.4 nm indicating minor fluctuations but relatively equilibrated state from 1 to 4 ns.

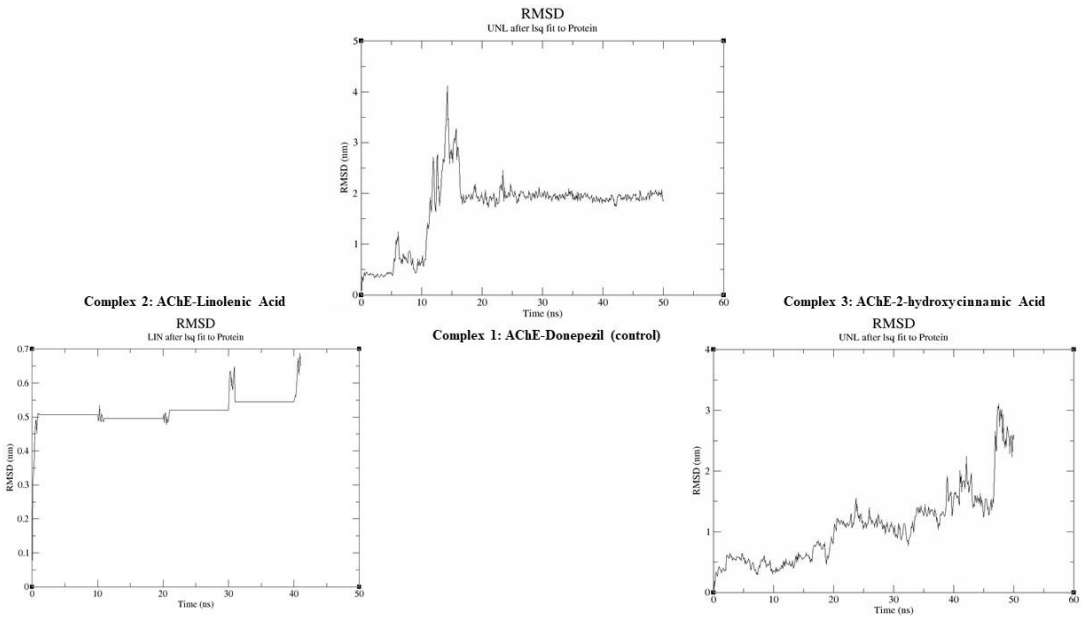

Figure 6: MD simulation interaction graphs of 50 ns trajectory depicting RMSD plot for complex of two lead phytochemicals Linolenic Acid and 2-hydroxycinnamic Acid along with standard control Donepezil with target protein AChE.

There is sudden peak in RMSD value, reading 0.64 at 4.7 ns indicating structural transition. These fluctuations gradually increase as the time proceeds toward 50 ns showing maximum peak in RMSD values at 46.8 to 48 ns, suggesting dynamic state of the complex 3. A compilation of plot of the RMSD graphical representation for all the three complexes is shown in **Fig. 7.**

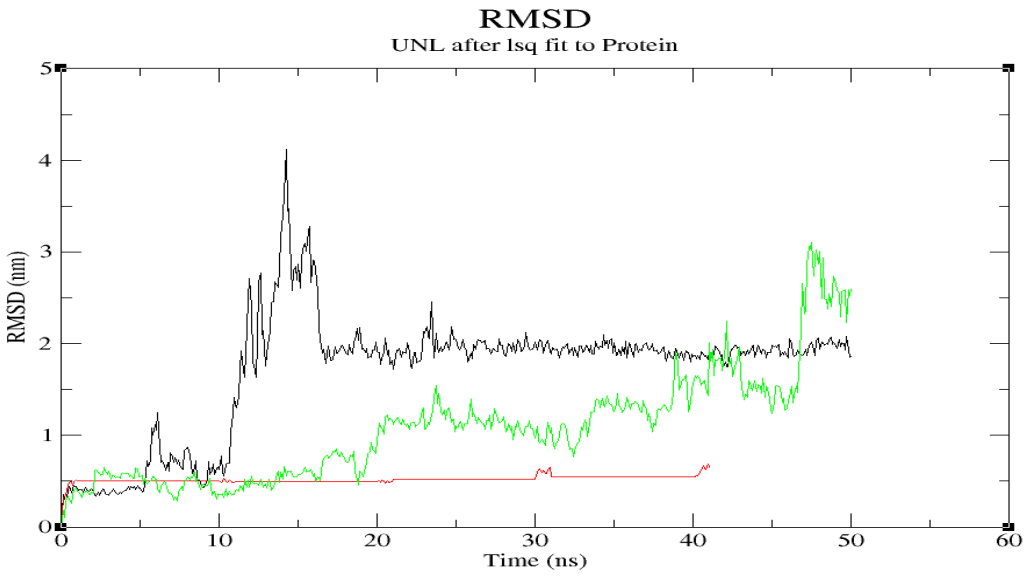

Figure 7: Combined RMSD analysis of target protein coupled with Donepexil (black), Linolenic Acid (Red) and 2-hydroxycinnamic Acid (Green) as a complex.

4.4 Analysis of Root-Mean-Square Fluctuation (RMSF)

To evaluate the area of protein which shows maximum fluctuation of residues over simulation trajectory is based peaks of RMSF. It is calculated using a command "gmx rmsf" in GROMACS. RMSF of individual amino acid of the protein evaluated in the duration of 50 ns MD simulation determines the flexibility of the protein system [59]. Residues/atoms with low RMSF values are suggestive maintaining stable integrity of the complex. Which helps in evaluation of impact of the ligand on the dynamics of protein. RMSF for the complexes shown in **Fig. 8.** show that the C terminal region show the in complex 1 and complex 3 in comparison to other regions in the protein. Whereas for complex 2 there is significant distribution of fluctuation in the atoms throughout the protein structure. In complex 1 the highest fluctuation towards the C-terminal reads to the

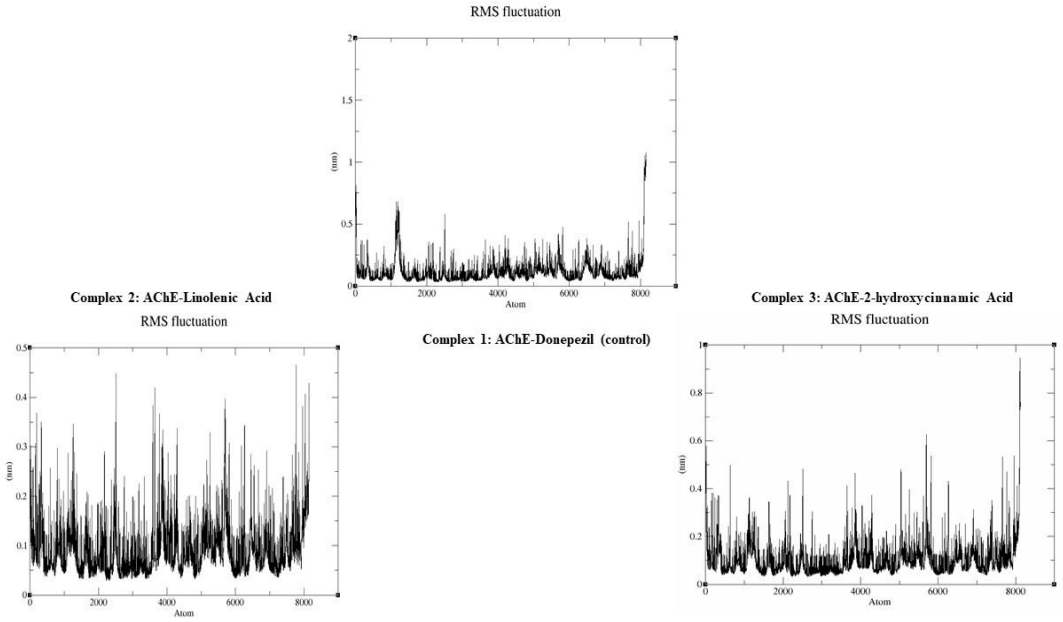

Figure 8: MD simulation interaction graphs of 50 ns trajectory depicting RMSF plot for complex of two lead phytochemicals Linolenic Acid and 2-hydroxycinnamic Acid along with standard control Donepezil with target protein AChE.

RMSF value in the range of 1.0-1.2 nm. While for complex 3, highest peak of RMSF value towards C-terminal atoms is between 7900-8000, lies in range of 0.8 to 1 suggestive of the stable integrity of protein towards C-terminal. For complex 2 the highest RMSD

value which are depicted by multiple peaks throughout the protein, lies between the range of 0.4 to 0.5 nm, concluding its integrity and stability. RMSF plot for all the three complexes are compiled in **Fig. 9.**

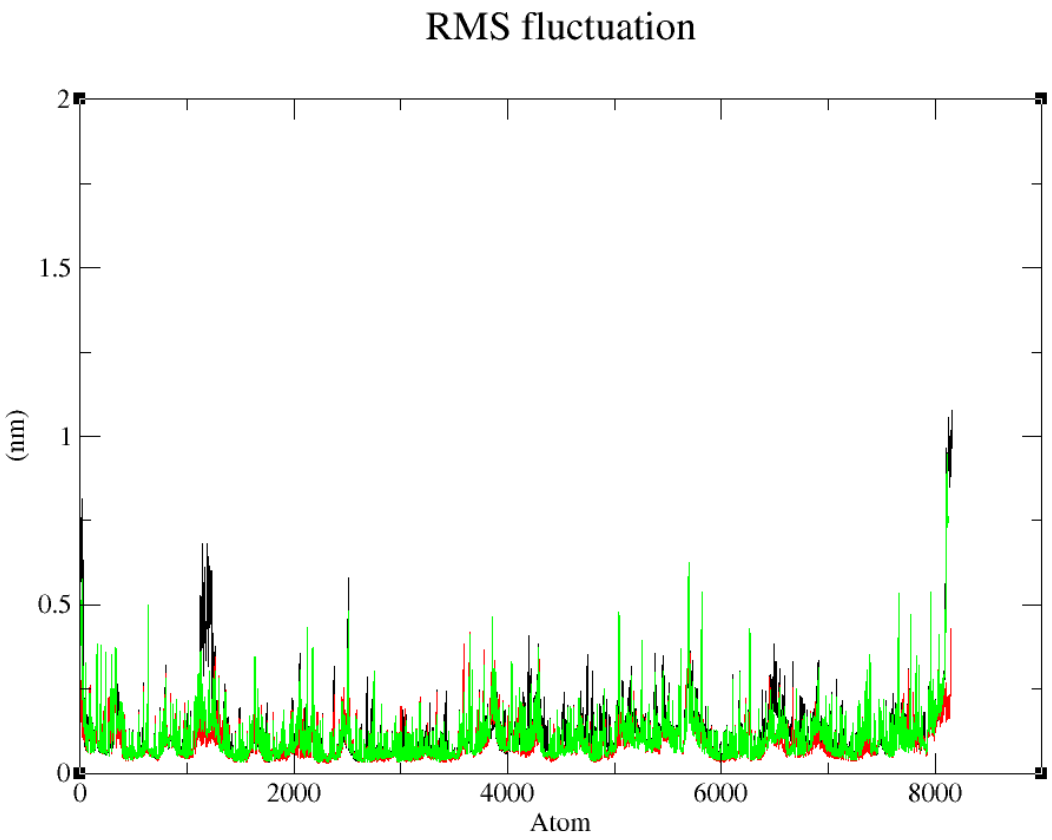

Figure 9: Combined RMSF analysis of target protein coupled with Donepexil (black), Linolenic Acid (Red) and 2-hydroxycinnamic Acid (Green) as a complex.

4.4 Analysis of Radius of Gyration (Rg)

This graphical data generated using command "gmx gyrate" in GROMACS, represents Radius of Gyration (Rg) values at different time stamps (in ps) during the 50 ns simulation. The information that can be extracted from Rg values tells us about the compactness of the complexes. Rg values are calculated with respect to three axis X, Y, Z providing insights on three directional distribution of the system. The Rg value of complex are 2.27 nm, 2.24 nm and 2.25 nm at 0 ps for complex 1, complex 2 and complex 3 respectively as depicted in **Fig. 10.** Average radius of gyration for complex 2 for the X, Y, Z axis are near about 1.68 nm, 1.85 nm and 2.03 nm respectively. Rg for complex

1starts from 2.27 at 0 ps and lies in the range of 2.28 nm to 2.43 nm for the rest of duration until 50000 ps. While Rg for complex 3 lies in the range of 2.25 nm to 2.314 nm from the duration 0 ps to 50000 ps. The results show narrow range of Rg value which can confer to stable conformations. Constants plateau in the graph are reflective of equilibrium conformations and stability of the complexes.

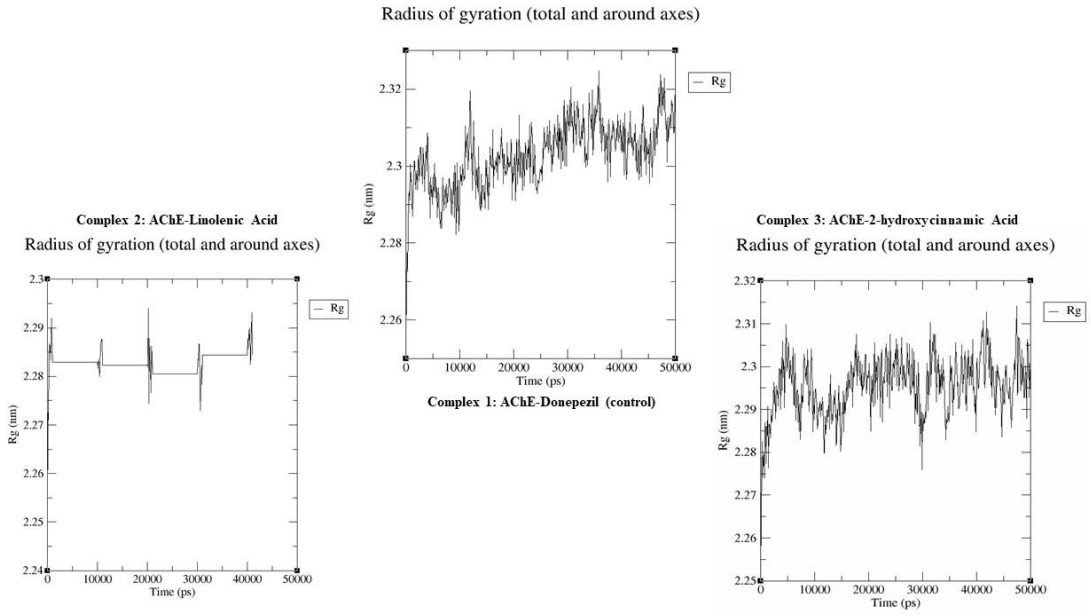

Figure 10: Compactness of the three docked phytochemical against AChE evaluated by Radius of Gyration

CHAPTER 5: DISCUSSION

Plants have traditionally been utilized as medicine to treat a spectrum of diseases all around the world. Not only is it an indispensable part of certain cultures and traditions, its availability, affordability and accessibility make these medicinal plants integral in identifying new therapeutic approaches. Richness in secondary metabolites like flavonoids, phenolic compounds, alkaloids etc synthesized by these plant as a defence mechanism also turn out to implicate medicative properties.

Alzheimer's disease is globally the leading cause of dementia, and presents a challenge in therapeutic research. AChE, a highly selective cholinesterase, plays a central role in reducing cholinergic transmission by virtue of its enzymatic activity. AChE hydrolyses upto 25000 ACh molecules per second, by breaking it down to acetate and choline, and thus decreasing the ACh levels in the brain. Patients in the progressed stages of Alzheimer’s disease have shown significantly decreased AChE levels, as low as 67%; on the other hand, a rise in the BChE levels of up to 120% of the normal level is also seen [2]. This finding suggests that the decreased levels of AChE are compensated by an increase in the levels of BChE by hydrolysing ACh. Hence, utilizing cholinesterase inhibitors can stop the hydrolysis of acetylcholine present in the synaptic cleft and as a result improve cholinergic transmission.

Existing research on AChE inhibition focuses on synthetic drugs, however a recent shift towards identifying novel candidates from natural resources for drug production has been observed. This is driven by the need to improve efficacy and decrease toxicity and side effects of potential natural compounds, owing to their natural origins. It is thus essential to identify efficacious cholinesterase inhibitors for drawing up better treatment regimen for AD.

Molecular docking is one method that greatly helps in the identification of such inhibitors. It works by using specific algorithms to find the active site of the receptor, in this case AChE, followed by the formation of binding pocket for the ligands. The docking score determines the binding affinity between the receptor (AChE) and the receptor, wherein lower docking scores indicate higher affinity.

Molecular docking studies have revealed that rutin, a flavone, shows higher affinity to both AChE and BChE, with respect to galantamine [60].

In this study we have shown that the best docked *caparris decidua* phytocompounds such as Linolenic Acid and 2-hydroxycinnamic Acid express better binding affinity when compared to their approved inhibitor of AChE, Donepezil. More interestingly, it was discovered that the binding affinity of both these phytochemicals, Linolenic acid and 2-hydroxycinnamic Acid showed higher affinities to AChE -7.8 Kcal/mol and -7.1 Kcal/mol respectively while the control Donepezil has the affinity of -7.0 Kcal/mol.

Further analysis revealed two promising compounds for drug development, Linolenic Acid (-7.8 Kcal/mol) and 2-hydroxycinnamic Acid (-7.1 Kcal/mol). They align with subsidiary parameters such as ADME analysis (absorption, distribution, metabolism and excretion), bioactivity and blood brain barrier permeability (BBB), which is essential for drugs targeting the brain. A Machine Learning-based prediction tool, SwissTargetPrediction, was also used in this study to illustrate the downregulation of a potent target molecule, PARP-1, using the same library of phytochemicals derived from *Capparis decidua.*

The stability of the structure, fluctuations behaviour and the conformational dynamics of the complex molecular system is studied using MD simulations in AD. Critical parameters to assess the stability of the system over time includes evaluation of RMSD, RMSF, Rg etc plots. Additionally, MD simulations aids in investigation of permeation of drugs through BBB, which is a step of utmost importance while targeting disorders related to central nervous system (CNS).

Overall *in-silico* analysis is an effective approach to elucidate the underlying molecular process of AD. It also has many advantages such as fast results, systematic preclinical screening of potential candidates and cost effectiveness. The study's objective of identifying natural sources to slow down the progression of Alzheimer's disease by inhibiting AChE, highlights its innovative approach [61].

Other than studies on acetylcholinesterase inhibitors, ongoing research is also focusing on discovering other treatment modalities for managing Alzheimer's disease. An example of one such approach is regulating Ach levels by inhibiting the activity of BChE, which in turn reduces choline uptake and induces muscarinic (M2) receptors. Approaches like these utilize feedback regulation, synaptic plasticity and neuronal excitability to combat the dysregulated cholinergic system.

Mechanisms involved in the pathogenesis of AD is also being targeted in several other approaches such as oxidative stress pathways, inflammatory pathways (specifically NFκB), aggregation of tau proteins, hyper phosphorylation and β and γ secretases activity, which processes amyloid precursor protein (APP) [62]. Modification of the said pathways are a promising prospect for discovering new treatments of AD. Some noteworthy potential targets shown by antioxidants for managing oxidative stress and the β and γ secretase inhibitors which can prevent amyloid plaque formation. Targeting these pathophysiological processes can slow down or even stop the progression of the disease [63].

Scouting for unconventional treatment methods gives hope for more effective regimens and enhances the possibility of combating the disease. It is paramount to such studies to assess the efficacy and safety of these alternative treatments, allowing for extensive options in treating AD. In order to advance our understanding and find potential treatments for this disease, further research and clinical trials must be encouraged.

CHAPTER 6: CONCLUSION

This study focuses on the inhibitory potential of certain phytochemicals extracted from *Capparis decidua*, Linolenic Acid and 2-hydroxycinnamic acid, against AChE enzyme for the treatment of Alzheimer's Disease. Upon evaluation of the drug likeness and pharmacokinetic properties of the phytochemicals, it was found that these compounds have characteristics which are favourable for drug development. However, in order to validate their therapeutic application, further in vitro and in vivo studies are necessary. For the most part though, significant activities were observed in these compounds which shows promise to improve the existing approach for the treatment of Alzheimer's disease.

Existing treatment options for Alzheimer's Diseases fall short with respect to their efficacy and thus leave scope for improvement. A potential alternative is the utilization of natural sources to inhibit AChE, which can halt the decline in cognitive functions due to impaired cholinergic transmission in patients of the disease. Utilization of advanced *in-silico* techniques like molecular docking and molecular dynamics simulation studies, has helped illustrate the bioactivity of phytochemicals derived from *Capparis decidua* against the pathological mechanisms of Alzheimer’s Disease.

Application of newer genomic and metabolomics technologies have the potential to improve the synthesis of functional neuroprotective drugs extracted from medicinal plants. Genome and transcriptome data can also be used to modify secondary metabolic pathways in plants.

The goal of this study is to discover an affordable and effective medication derived from natural substances, which can be used in treating the patients of Alzheimer’s disease worldwide. However, a research gap remains in this field, due to limited scientific evidence on the nutraceutical properties of herbal medicine, which should be explored and assessed in order to expand our understanding of the disease as well as improve our arsenal to combat the progression of the disease.

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DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi college of Engineering)

Bawana Road, Delhi-110042

CERTIFICATE

I hereby certify that the Project Dissertation titled "**Molecular Docking and Simulation Studies on phytochemical profile of *Capparis decidua* for inhibition of Acetylcholinesterase in AD**", which is submitted by Saleha Siddiqui, 2K21/MSCBIO/38, Delhi Technological University Delhi, in partial fulfilment of the requirement for the award of the degree of Masters in Science, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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Department of biotechnology

Delhi technological university

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PROF. PRAVIR KUMAR

HEAD OF DEPARTMENT

Department of biotechnology

Delhi technological university

Gmail saleha siddiqui <salehasiddiqui29@gmail.com>

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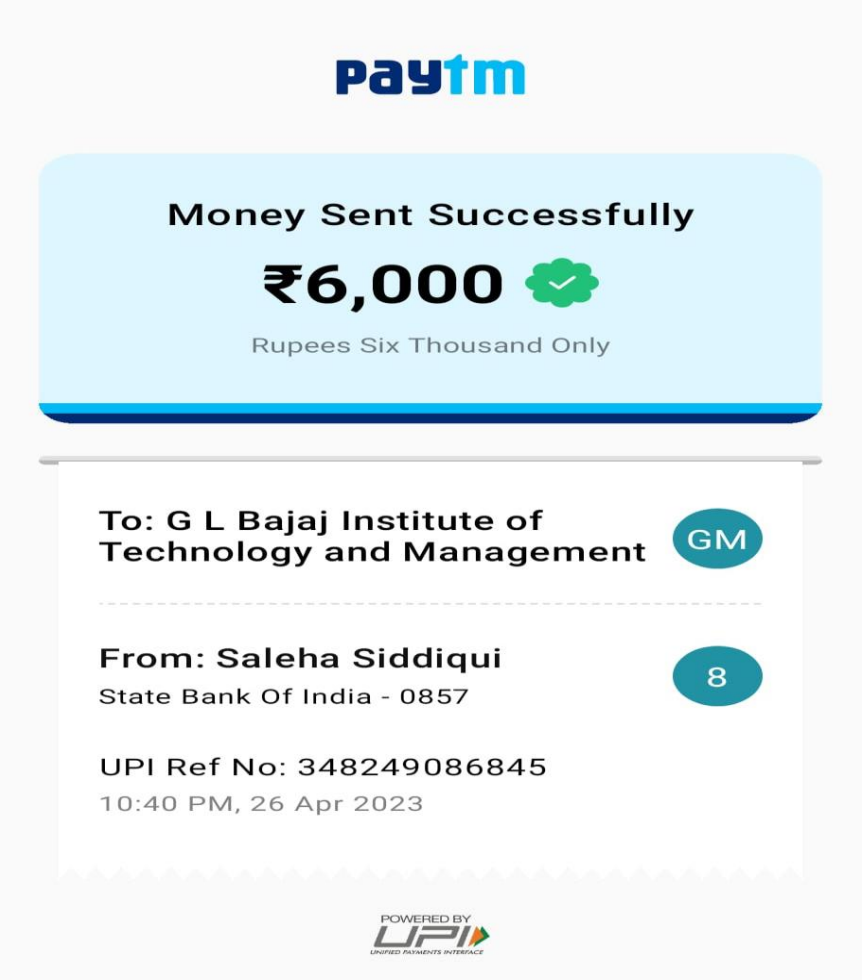

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